Comparative Study on Degradation, Aggregation and Rheological Properties of Actomyosin from Cold, Temperate and Warm Water Fish Species

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Abstract

Proteolytic degradation and thermal aggregation patterns and dynamic rheological properties of actomyosin prepared from Alaska pollock (*Theragra chalcogramma*), Pacific whiting (*Merluccius productus*), bigeye snapper (*Priacanthus* spp.), lizardfish (*Saurida* spp.) and threadfin bream (*Nemipterus* spp.) surimi were comparatively studied. There was a significant endogenous protease activity observed in crude actomyosin samples where Pacific whiting and lizardfish exhibited the highest proteolytic activity. SDS-PAGE analysis showed that intensity of myosin heavy chain bands of Pacific whiting, bigeye snapper, lizardfish and threadfin bream decreased with extended incubation time, resulting in medium and low molecular weight proteins. For all tested fish species, a $0.5°C$ min^{-1} heating rate resulted in higher turbidity values followed by $1.0°C$ min^{-1} and then $2.0°C$ min^{-1}. Temperature onset point for turbidity increase was significantly affected by species. Storage modulus peak temperatures, obtained from temperature sweep tests of actomyosins, were similar to thermal transition values obtained from differential scanning calorimetry, indicating that peaks obtained from the dynamic tests were related to protein denaturation temperatures. Slower heating rate shifted the thermal transition temperature to a lower value. These observations should give better understanding of the thermal sensitivity of fish species with regards to gelation properties and proteolytic degradation.

Keywords: Surimi, actomyosin, proteolytic degradation, denaturation, dynamic rheologic properties, DSC

Soğuk, Ilık ve Sıcak Su Türlerinden Elde Edilen Aktomiyosinin Parçalanma, Topaklanma ve Reolojik Özellikleri Hakkında Karşılaştırmalı Çalışma

Özet

Bu çalışmada, Alaska pollock (*Theragra chalcogramma*), Pacific whiting (*Merluccius productus*), bigeye snapper (*Priacanthus* spp.), lizardfish (*Saurida* spp.) ve threadfin bream (*Nemipterus* spp.) surimilerinden hazırlanan aktomyozinin proteolitik parçalanma ve ısıl topaklanma eğilimi ve reolojik özellikleri karşılaştırmalı olarak incelenmiştir. Arıtılmamış aktomyozin örneklerinde belirgin bir endojen proteolitik aktivite görülürken, Pacific whiting ve lizardfish en yüksek proteolitik aktiviteyi göstermişlerdir. SDS-PAGE analizi, Pacific whiting, bigeye snapper, lizardfish ve threadfin bream miyozin ağır zincir bantlarının yoğunluğunun inkübasyon süresi uzadıkça azaldığını, orta ve düşük molekül ağırlıklı proteinlerin oluştuğunu göstermiştir. Test edilen bütün balık türleri için $0,5°C$ dk^{-1} ısıtma hızı daha yüksek bulanıklık değerlerine neden olurken, bunu $1,0°C$ dk^{-1} ve daha sonra $2,0°C$ dk^{-1} ısıtma hızları izlemiştir. Bulanıklığın artmaya başladığı sıcaklık değeri balık türünden belirgin bir şekilde etkilenmiştir. Aktomyozinin sıcaklık tarama testlerinden elde edilen dinamik depolama modülü pik sıcaklıklarının diferansiyel taramalı kolorimetre geçiş sıcaklık değerlerine yakın olduğu görülmüştür. Bu, dinamik testlerden elde edilen piklerin protein denatürasyon sıcaklıkları ile ilgili olduğuna işaret etmektedir. Düşük ısıtma hızları, ısıl geçiş sıcaklığının daha düşük bir değere kaydırmıştır. Bu sonuçlar, protein jeli oluşum özellikleri ve proteolitik parçalanma açısından balık türlerinin ısıl hassasiyetinin daha iyi anlaşılmasını sağlayacaktır.

Anahtar Kelimeler: Surimi, aktomiyozin, proteolytik parçalanma, denatürasyon, dinamik reolojik özellikler, DSC.

Introduction

Maintaining functionality of myofibrillar proteins is of prime importance for muscle foods. Degradation and denaturation cause loss of protein functionality since it is closely associated with the integrity of proteins. Better understanding of thermal stability and temperature sensitivity of fish proteins

with proteolytic enzymes would help to optimize surimi and surimi seafood manufacturing and a timely intervention to maximize fish protein functionality.

Alaska pollock is the major fisheries resource used for surimi production. For the last decade, however, other fish species such as, Pacific whiting, threadfin bream, lizardfish and bigeye snapper, have also been introduced into surimi production. Proteolytic degradation of myofibrillar proteins presents a technical and operational problem for surimi from some of these fish species. While serine proteases were responsible for textural breakdown of threadfin bream (Toyohara and Shimizu, 1988) and bigeye snapper (Benjakul *et al.*, 2003a), cathepsin L, a cysteine proteinase, hydrolyzed Pacific whiting muscle proteins, causing severe textural degradation in whiting surimi (An *et al.*, 1994b).

Actomyosin is the major protein responsible for gelation, which plays an important role in the texture and processing characteristics of meat products (Ahhmed *et al.*, 2009). Heat-induced gelation of fish myofibrillar proteins occurs in three-steps; dissociation of the proteins in the presence of salt, unfolding of protein molecules due to heating and aggregation of unfolded protein domains via hydrogen and disulfide bonds and hydrophobic interactions (Stone and Stanley, 1992; Lefevre *et al.*, 1999). When aggregation is slow, with respect to denaturation, heat-denatured proteins align in an orderly fashion to form a fine gel network, resulting in a more elastic gel (Hermansson, 1979). The effect of heating rate on gelation properties of myofibrillar proteins has been well established (Foegeding *et al.*, 1986; Yongsawatdigul and Park, 1999; Sun and Arntfield, 2011). Their findings, however, were possibly influenced by the presence or absence of endogenous enzymes. Contrary to previous theory that slower heating produces better gels, Riemann *et al.* 2004 reported that cook value, rather than heating rate, ultimately determines gel properties.

The objectives of this study were to: (1) investigate proteolytic degradation patterns by determining the influence of autolytic activity at their respective optimum autolysis temperature; (2) investigate the influence of heating rate on thermal aggregation properties; (3) investigate thermal denaturation (i.e., endothermic transitions) and gelation (i.e., G') properties; (4) compare degradation, aggregation and dynamic rheological properties of several fish surimi using the same methods.

Materials and Methods

Materials

Surimi from five different species was used for this study. Alaska pollock (*Theragra chalcogramma*) was obtained, as a cold-water species, from UniSea (Seattle, WA, USA) and Pacific whiting (*Merluccius productus*), as a temperate-water species, from Pacific

Surimi (Warrenton, OR, USA). All warm-water species (bigeye snapper [*Priacanthus* spp.], lizardfish [*Saurida* spp.] and threadfin bream [*Nemipterus* spp.]), were provided by Andaman Surimi Industry (Bangkok, Thailand). All frozen surimi samples were high grade (FA) freshly produced and stored at –20°C or below. Frozen blocks were cut into small sub-blocks (1 kg) and vacuum-packed and kept at –30°C not more than two months before use.

Bovine serum albumin (BSA), sodium dodecyl sulphate (SDS), trichloroacetic acid (TCA), phenylmethanesulfonyl fluoride (PMSF) were purchased from Sigma Chemicals (St. Louis, MO, USA). All chemicals were of analytical grade.

Actomyosin Preparation

Actomyosin from surimi was prepared according to Yongsawatdigul and Park, 2003 with slight modifications. The preparation of samples was carried out at 0-4°C to minimize proteolysis. Surimi was homogenized with 20 mM cold phosphate buffer (pH 7.0) containing 50 mM KCl and 0.05 mM PMSF at a 1:9 ratio in a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA) for 2 min. The homogenate was centrifuged at $5000 \times g$ for 30 min at 4°C to remove cryoprotectants and other soluble materials. The precipitate was collected as crude actomyosin and used as a sample throughout the study. Protein concentration of actomyosin was determined by Lowry's assay (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard.

Autolysis

Optimum temperature for proteolysis was determined by conducting autolysis at 25, 30, 40, 50, 55, 60, 65, 70 and 75°C for 90 min. A time course study was conducted at the maximum autolysis temperature once the optimum autolysis temperature was determined. Three grams of crude fish actomyosin were incubated at the maximum autolysis temperature for 0, 30, 60, 120, 180 and 240 min, respectively. Autolysis was stopped by adding 27 ml 5% cold TCA solution. The mixture was incubated at 4°C for 15 min and centrifuged at $6000 \times g$ for 15 min. The TCA soluble proteins were analyzed for the oligopeptide content using Lowry's assay and expressed as $\mu mol\ ml^{-1}$ of tyrosine released (An *et al.*, 1994b). Sample blanks were kept on ice and analyzed to correct for oligopeptide present in fish muscle.

Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE samples were prepared as described by Esturk *et al.*,2004. Retention of crude actomyosin, which was incubated at the maximum autolysis temperature and sampled at various time intervals, was investigated using SDS-PAGE as described by

Laemmli 1970 Stacking gels and separating gels were prepared at 4% and 10% (w v^{-1}) polyacrylamide gel, respectively. SDS-PAGE gel was stained in 0.125% Coomassie blue R-250 (Bio-Rad, Richmond, CA, USA) and destained in 10% methanol and 10% acetic acid. A wide range molecular weight standard mixture (Sigma Chemical Co., St. Louis, MO, USA) was used, which included myosin from rabbit muscle (205 kDa), β-galactosidase from *E. coli* (116 kDa), phosphorylase b from rabbit muscle (97 kDa), fructose-6-phosphate kinase of rabbit muscle (84 kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase (55 kDa), chicken egg ovalbumin (45 kDa), glyceraldehydes-3-phosphate dehydrogenase of rabbit muscle (36 kDa), carbonic anhydrase of bovine erythrocyte (29 kDa) and bovine trypsinogen (24 kDa).

Turbidity

Crude actomyosin precipitate was diluted to 0.5 g l^{-1} in 0.6 M KCl, 20 mM This-HCl buffer, pH 7.0 and heated from 10 to 80°C at 0.5, 1.0 or 2.0°C min^{-1} heating rates. A programmable water bath (Neslab, Model RTE 100LP, Portsmouth, NH, USA) was connected to an UV-VIS spectrophotometer (UV–2401 PC, Shimadzu Scientific Instruments Inc., Baltimore, MD, USA) to heat the samples. Protein solution was placed into a quartz cell (light path length 10 mm). Protein aggregation was determined by measuring absorbance at 320 nm (Xiong and Blanchard, 1994).

Oscillatory Dynamic Rheology

Development of an actomyosin gel network was measured as a function of temperature using a CS-50 rheometer (Bohlin Instruments, Inc., Cranbury, NJ, USA). Actomyosin samples were placed between the cone and plate (CP-4/40). A plastic cover (trapper) with a moistened sponge inside was used to prevent sample drying during heating. The sample was heated from 10 to 90°C at a heating rate of 0.5 and 1.0°C min^{-1}.

Micro Differential Scanning Calorimetry (Micro DSC)

DSC studies were performed using a SETARAM micro differential scanning calorimeter (SETARAM Co., Lyon, France). Temperature calibrations were performed using naphthalene. Samples, approximately 500 mg, were accurately measured in a standard stainless steel vessel. Samples were scanned at 0.5 and 1.0°C min^{-1} heating rates over a range of 10 to 90°C.

Statistical Analysis

Two different lots of surimi were used for each species. Triplicate measurements were taken for each analysis and data were averaged over the three measurements. The experimental data were analyzed by using one-way ANOVA and mean comparison using Duncan's multiple range test at 95% confidence level (P≤0.05). The statistical software used was SAS for Windows (version 8.02, SAS Institute, Cary, NC, USA).

Results and Discussion

Optimum Time and Temperature of Proteolysis

While proteolysis of Alaska pollock (AP), lizardfish (LF) and Pacific whiting (PW) crude actomyosin did not occur until the temperature >25°C, for bigeye snapper (BS) and threadfin bream (TB), it was not observed until the temperature >40°C. Then, it gradually increased and reached a maximum at 55, 55, 65, 60 and 65°C for AP, PW, BS, LF and TB, respectively (Figure 1a). Heat stable endogenous proteinases, primarily hydrolyses myosin, cause textural softening upon heating. Similar maximum proteolysis temperatures were reported in
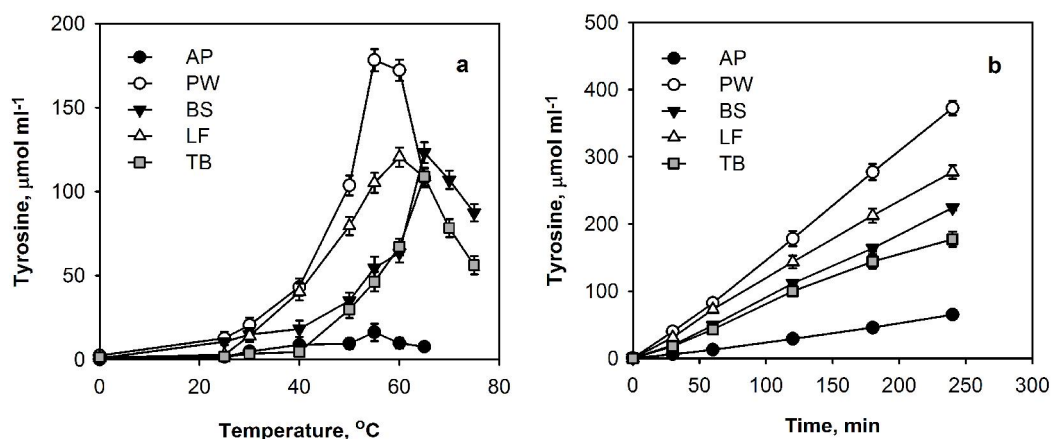

Figure 1. Autolytic activity (a) as a function of temperature after 90 minute incubation, and (b) as a function of incubation time at the optimal autolysis temperature of Alaska pollock (AP), Pacific whiting (PW), bigeye snapper (BS), lizardfish (LF) and threadfin bream (TB) at 55, 55, 65, 60 and 65 °C, respectively. Values are given as mean ± SD, n= 3.

literature. An *et al.,* 1994a reported that cathepsin L, a cysteine proteinase, is responsible for the textural degradation of PW and has a maximum activity at 55°C. Similarly, heat-stable proteases were found to be responsible for the textural breakdown of BS (Benjakul *et al.,* 2003a), LF (Benjakul *et al.,* 2003b; Yongsawatdigul and Piyadhammaviboon, 2004) and TB (Toyohara and Shimizu, 1988) exhibiting their maximum activity at 60, 60, 65°C, respectively.

Crude actomyosin samples showed a significant proteolytic activity which linearly increased with incubation time at maximum proteolysis temperatures throughout the time-course experiment (Figure 1b). This indicated that washing process during surimi production did not completely remove the proteases in samples, which may adversely affect the overall gel quality. PW had the highest proteolytic activity, followed by LF, BS, TB and AP, in descending order, respectively.

SDS-PAGE

AP crude actomyosin sample incubated at 55 °C did not exhibit significant proteolytic activity and the myofibrillar proteins remained almost unchanged. However, a different protein band (116<Mw<205 kDa) was observed and its intensity increased as incubation was prolonged, indicating the presence of a minimum level of proteolytic degradation for AP even at high grade (FA) (Figure 2a).

PW, incubated at 55 °C, exhibited the most severe degradation of myosin heavy chain (MHC), which was significantly noticeable after 30 min of incubation (Figure 2b). However, compared to MHC, degradation of actin was slow and minimal. A decrease in the intensity of MHC and actin resulted in the subsequent appearance of medium molecular weight proteins around 100 kDa and low molecular
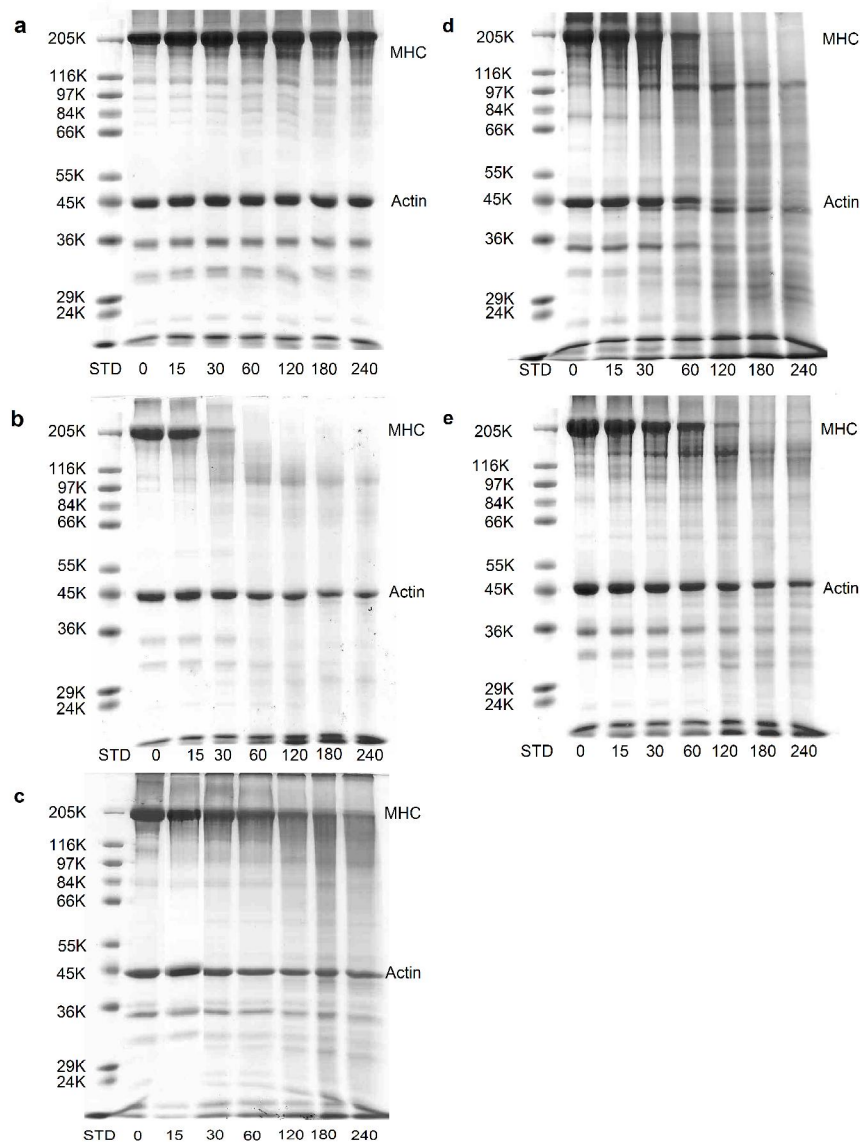

Figure 2. SDS-PAGE patterns of actomyosin from Alaska pollock (a), Pacific whiting (b), bigeye snapper (c), lizardfish (d) and threadfin bream (e) surimi incubated at 55, 55, 65, 60 and 65 °C, respectively. STD: wide range molecular weight standard, 0-240 incubation times in min.

weight proteins below 24 kDa. (An *et al.*, 1994b) studied the autolytic pattern of PW surimi and reported that MHC band intensity was substantially reduced within 5 min incubation at 55°C and completely disappeared within 20 min. In addition, MHC and actin were extensively hydrolyzed when PW surimi was slowly heated at a rate of 1.0°C min^{-1} from 10 to 90°C (Yongsawatdigul and Park, 1996).

Degradation of MHC was noted for all warm water fish species (BS, LF and TB) as incubation time increased (Figure 2c, 2d, 2e). LF, incubated at 60°C, exhibited the most significant proteolytic activity (Figure 2d) among warm water fish species studied followed by TB (Figure 2e). Similar to PW, intensity of MHC and actin bands of BS, LF and TB decreased with extended incubation time, resulting in medium molecular weight proteins (66 kDa< Mw <205 kDa) and low molecular weight proteins (<45 kDa).

The extent of proteolysis varied with fish species. AP exhibited the minimum level of proteolytic degradation followed by BS among the species tested. MHC was the primary target for proteolytic degradation. MHC of PW exhibited the

fastest hydrolysis compared to the other fish species and it completely disappeared after 1 h of incubation (Figure 2b). It was also noted that LF exhibited the most significant actin hydrolysis (Figure 2d). Differences in degradation patterns indicated that the type of proteases (serine or cysteine) may play a role in the hydrolysis rates of MHC and actin (Benjakul *et al.*, 2003b).

Turbidity

Initially, absorbance values remained fairly constant until reaching the onset temperature, where absorbance started to increase. Statistical analysis revealed that until reaching the onset temperature, the heating rate did not have a significant effect (P>0.05) on the absorbance values of any fish species (Figure 3). Absorbance of actomyosin solutions at 320 nm increased with temperature due to the formation of protein aggregates. All fish species followed a similar trend.

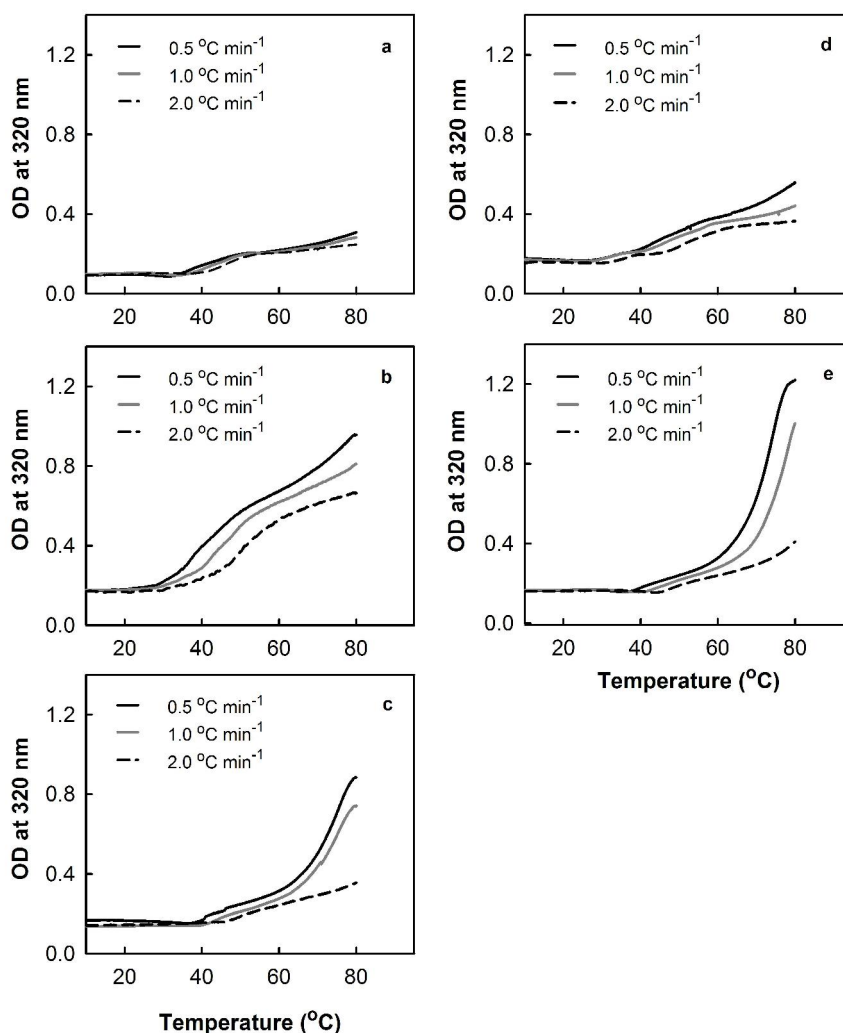The onset temperature appeared to be dependent on heating rates. Slower heating rates were related to

Figure 3. Changes in turbidity of actomyosin solutions (0.5 g l^{-1} in 0.6 M KCl, 20 mM Tris-HCl buffer, pH 7.0) from Alaska pollock (a), Pacific whiting (b), bigeye snapper (c), lizardfish (d) and threadfin bream (e). Samples were heated linearly at 0.5, 1.0, 2.0°C min^{-1} at 320 nm.

lower onset temperatures. The onset temperatures of the samples heated at 0.5, 1.0 and 2°C min^{-1} heating rates were 31.9, 34.5 and 37.2°C for AP (Figure 3a), 27.8, 29.2 and 30.6°C for PW (Figure 2b), 38.0, 40.2 and 46.7°C for BS (Figure 2c), 25.6, 27.2 and 30.4°C for LF (Figure 2d) and 38.6, 40.5 and 45.5°C for TB (Figure 2e), respectively.

After reaching the onset temperature, absorbance readings increased rapidly with temperature. Similar results were reported for myofibrillar proteins from herring (Chan *et al.*, 1993), cod (Chan *et al.*, 1993; Yongsawatdigul and Park, 1999), chicken (Xiong and Blanchard, 1994), a mixture of cod and herring (Chan and Gill, 1994) and Atlantic salmon (Lefevre *et al.*, 2007). Gill *et al.*,1992 demonstrated that an increase in absorbance of heated fish myosin correlated with the formation of myosin aggregates.

Thermal aggregation of AP, PW, BS, LF and TB actomyosin, measured by turbidimetry, appeared to be a continuous phenomenon for which the increase was linear up to 80°C. For all fish species, there was a transitional shift in absorbance readings around 60°C. This could be due to the formation of larger aggregates as a result of myosin aggregation. Heating rate also affected the magnitude of absorbance values for all fish species. A 0.5°C min^{-1} heating rate resulted in the highest turbidity values followed by 1°C min^{-1} and then 2°C min^{-1}. Higher absorbance readings at slower heating rates were probably due to

the formation of larger aggregates during respectively longer time.

The functional characteristics of food proteins are influenced by heating time and temperature as well as heating rate. Depending upon the molecular properties of proteins in the unfolded state, it undergoes one of two types of interactions. Proteins containing a high level of apolar amino acid residues undergo random aggregation via hydrophobic interactions, resulting in an opaque coagulum or precipitate. On the other hand, proteins containing below a critical level of apolar amino acid residues form soluble aggregates, which set into a thermally reversible, transparent gel network upon cooling (Damodaran, 1997). When the heating conditions are extreme, protein molecules may not have time to align themselves in an ordered fashion.

Oscillatory Dynamic Rheology and Micro Differential Scanning Calorimetry (Micro DSC)

Storage modulus (G') and phase angle of actomyosin samples at 0.5 and 1.0°C min^{-1} heating rates were measured using a temperature sweep (TS) mode (Figure 4). There was a insignificant effect of heating rate on G' and phase angle until reaching the second G' transition temperature (the lowest G'). The same trend was observed for all fish species tested. However, G' of 0.5°C heating rate was significantly
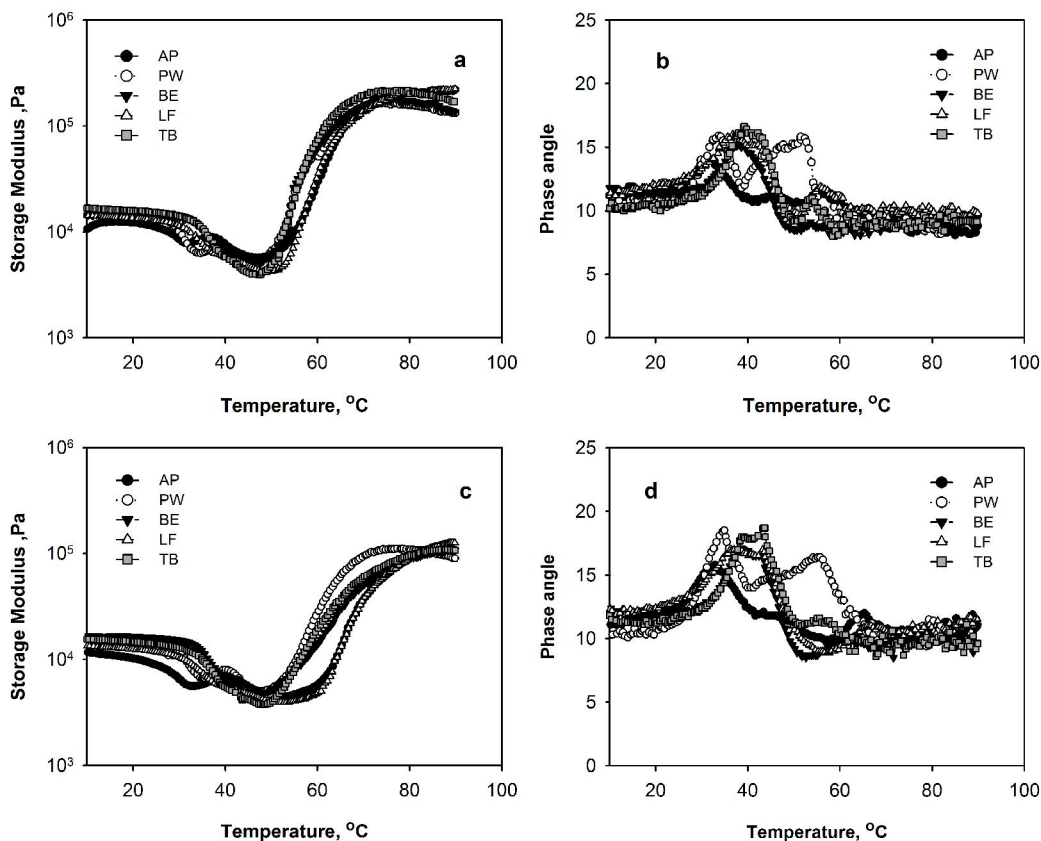

Figure 4. Changes in storage modulus (G') and phase angle of actomyosin from Alaska pollock, Pacific whiting, bigeye snapper, lizardfish and threadfin bream surimi during heating from 10 to 90°C at a rate of 0.5 (a, b) and 1°C min^{-1} (c, d). AP, Alaska pollock; PW, Pacific whiting; BS, bigeye snapper; LF, lizardfish; TB, threadfin bream.

higher than that of 1.0°C after that transition temperature.

The storage modulus (G') decreased and phase angle increased for all samples around at 30°C possibly due to the destabilization of polar bonding in the myosin tail region, resulting in increased mobility of the molecule Thermal denaturation temperature for myosin was the lowest for AP (~33°C), followed by PW (~34-35°C), BS (~38°C), LF (~37-38°C) and finally TB (~39-40°C). These results suggested that the stability of myosin from different fish species is related to the environmental temperature at which fish live and supported the general statement that proteins of warm water species were more thermostable than those of cold or temperate water species (Sano *et al.*, 1990). In concurrence with the rheological changes, the DSC thermograms of crude actomyosin samples resulted in endothermic peaks at 33-40°C for all samples, confirming that myosin contributes to the first peak in G' values observed in the temperature sweep (Table 1). Hastings *et al.,* 1985 and Howell *et al.,* 1991 reported similar findings supporting our results.

After a slight increase, the storage modulus (G') continued to decrease, reaching the lowest values around at 46 to 50°C depending on the fish species. This may be attributed to the unfolding of light meromyosin (tail) (LMM) and heavy meromyosin (HMM) chains (Sano *et al.*, 1988; Sano *et al.*, 1989). Endothermic peaks obtained from DSC thermograms and the G' peaks obtained from rheograms were close for BS and TB, however, those peaks for AP and PW were quite different. Warm water fish species had higher thermal transition (Tm) values than cold or temperate water species for myosin subfragments.

G' increased substantially after 50°C, possibly due to myosin aggregation. The DSC thermograms of crude actomyosin samples showed an endothermic region around 60-67°C, which corresponds to the

transitions due to the denaturation of actin. Hemung *et al.*, 2008 found that Tm values of actin for PW and TB natural actomyosin samples were 75.3 and 78.4°C, respectively, which were different than our findings (Table 1). This difference might be due to a difference in sample preparation techniques, including different buffer solutions used.

There were 3 major endothermic peaks clearly distinguishable for all fish species from DSC measurements and 2 transition peaks from the temperature sweep of the dynamic rheology test. The multiple transitions of fish species studied in the present study suggested the independent structural changes of myosin subfragments and actin. Myosin thermal denaturation is known to occur on several structural domains capable of undergoing independent transitions (Privalov, 1982) depending on the biological source and experimental environment (pH, ionic strength). Saeed and Howell, 2004 studied the effect of storage temperature on DSC thermograms of Atlantic mackerel and found that Tm values decreased significantly for samples stored at –20°C compared with those stored at –30°C after 2 years. Fernandez-Martin *et al.*, 1998 reported Tm temperatures of blue whiting were 33.5, 44.5 and 74.7°C. These peaks reflected the thermal response of myosin (subfragment S1), myosin rod and actin, respectively.

Tm of myosin and actin for various fish species are reported in literature (Hastings *et al.*, 1985; Poulter *et al.*, 1985; Park and Lanier, 1989; Togashi *et al.*, 2002; Park *et al.*, 2008). Tm values for myosin varied within the range of 29 to 59°C. A narrower temperature range, however, was reported for actin (65-75°C).

Conclusions

Myosin and actin were extensively hydrolyzed by endogenous proteases for all species except AP.

Table 1. Differential scanning calorimetry (DSC) and temperature sweep (TS) peak temperatures of crude actomyosin from Alaska pollock, Pacific whiting, bigeye snapper, lizardfish, and threadfin bream at 0.5 and 1.0 °C min^{-1} heating rates

Fish Species	Application	Peak 1 (°C)		Peak 2 (°C)		Peak 3 (°C)	
		0.5 °C min^{-1}	1.0 °C min^{-1}	0.5 °C min^{-1}	1.0 °C min^{-1}	0.5 °C min^{-1}	1.0 °C min^{-1}
Alaska pollock	DSC	32.91[de]	33.63[d]	38.48[f]	41.78[c]	64.47[a]	67.26[a]
	TS	32.80[e]	33.30[d]	47.60[bc]	48.20[b]	–	–
Pacific whiting	DSC	34.15[de]	35.16[c]	39.32[f]	41.59[c]	62.07[b]	64.60[b]
	TS	34.30[d]	35.40[c]	44.20[e]	48.20[b]	–	–
Bigeye snapper	DSC	37.85[bc]	38.8[ab]	45.52[de]	47.76[b]	61.00[bc]	62.85[c]
	TS	38.60[ab]	38.70[ab]	46.60[d]	48.30[b]	–	–
Lizardfish	DSC	36.98[c]	37.73[b]	46.08[cde]	47.87[b]	59.75[c]	61.60[d]
	TS	37.80[bc]	38.80[ab]	50.20[a]	51.60[a]	–	–
Threadfin bream	DSC	39.26[a]	39.26[ab]	48.31[b]	48.31[b]	60.30[bc]	60.30[d]
	TS	39.40[a]	39.90[a]	47.60[bc]	48.60[b]	–	–

[a-f]Different superscripts in the same column indicate significant differences (P≤0.05)

Aggregation, thermal denaturation and dynamic rheological properties of actomyosin were greatly affected by fish species and the heating rate. A slower heating rate resulted in higher turbidity and lower temperature onset points for turbidity increase compared to a faster heating rate. Similarly, lower thermal transition temperatures measured in oscillatory dynamic rheometer and DSC for fish muscle proteins that were related to a shift to lower temperature in denaturation and aggregation processes for the fish species studied. Myosin denaturation temperature was species dependent and cold or temperate water species (AP, PW) had lower Tm values than temperate water species (BS, LF, TB). During the production of surimi and surimi seafood, it is important to know thermal aggregation and proteolytic degradation properties of fish proteins for the proper use of surimi. As a step for ensuring fish protein quality, surimi processing guidelines should be specifically determined for each particular fish species based on their respective thermal stability and proteolytic activity.

Acknowledgments

References

Ahhmed, A.M., Nasu, T., Huy, D.Q., Tomisaka, Y., Kawahara, S. and Muguruma, M. 2009. Effect of microbial transglutaminase on the natural actomyosin cross-linking in chicken and beef. Meat Science, 82:170-178.

An, H.J., Seymour, T.A., Wu, J.W. and Morrissey, M.T. 1994a. Assay systems and characterization of Pacific whiting (*Merluccius productus*) protease. Journal of Food Science, 59:277-281.

An, H.J., Weerasinghe, V., Seymour, T.A. and Morrissey, M.T. 1994b. Cathepsin degradation of Pacific whiting surimi proteins. Journal of Food Science, 59:1013-1017.

Benjakul, S., Visessanguan, W. and Leelapongwattana, K. 2003a. Purification and characterization of heat-stable alkaline proteinase from bigeye snapper (*Priacanthus macracanthus*) muscle. Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology, 134:579-591.

Benjakul, S., Visessanguan, W. and Tueksuban, H. 2003b. Heat-activated proteolysis in lizardfish (*Saurida tumbil*) muscle. Food Research International, 36:1021-1028.

Chan, J.K. and Gill, T.A. 1994. Thermal aggregation of mixed fish myosins. Journal of Agricultural and Food Chemistry, 42:2649-2655.

Chan, J.K., Gill, T.A. and Paulson, A.T. 1993. Thermal aggregation of myosin subfragments from cod and herring. Journal of Food Science ,58:1057-1061.

Damodaran, S. 1997. Food proteins and their applications. NY: Marcel Dekker Inc, New York 681pp.

Esturk, O., Park, J.W. and Thawornchinsombut, S. 2004. Effects of thermal sensitivity of fish proteins from various species on rheological properties of gels. Journal of Food Science, 69: 412-416.

Fernandez-Martin, F., Perez-Mateos, M. and Montero, P. 1998. Effect of pressure/heat combinations on blue whiting (*Micromesistius poutassou*) washed mince: thermal and mechanical properties. Journal of Agricultural and Food Chemistry, 46:3257-3264.

Foegeding, E.A., Allen, C.E. and Dayton, W.R. 1986. Effect of heating rate on thermally formed myosin, fibrinogen and albumin gels. Journal of Food Science, 51:104-108.

Gill, T.A., Chan, J.K., Phonchareon, K.F. and Paulson, A.T. 1992. Effect of salt concentration and temperature on heat-induced aggregation and gelation of fish myosin. Food Research International, 25:333-341.

Hastings, R.J., Rodger, G.W., Park, R., Matthews, A.D. and Anderson, E.M. 1985. Differential scanning calorimetry of fish muscle - the effect of processing and species variation. Journal of Food Science, 50:503-506.

Hemung, B.O., Li-Chan, E.C.Y. and Yongsawatdigul, J. 2008. Thermal stability of fish natural actomyosin affects reactivity to cross-linking by microbial and fish transglutaminases. Food Chemistry, 111:439-446.

Hermansson, A.M. 1979. Aggregation and denaturation involved in gel formation. In: A Pour-El, (Ed), Functionality and Protein Structure. American Chemical Society, Washington, DC. 81-103.

Howell, B.K., Matthews, A.D. and Donnelly, A.P. 1991. Thermal-stability of fish myofibrils -a differential scanning calorimetric study. International Journal of Food Science and Technology 26:283-295.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680 - 685.

Lefevre, F., Culioli, J., Joandel-Monier, S. and Ouali, A. 1999. Muscle polymorphism and gelling properties of myofibrillar proteins from poultry, mammals and fish. In: Y. L., Xiong, C-T., Ho, F., Shahidi, (Eds), Quality Attributes of Muscle Foods. Kluwer Academic/Plenum Publishers, New York. 365–391.

Lefevre, F., Fauconneau, B., Thompson, J.W. and Gill, T.A. 2007. Thermal denaturation and aggregation properties of Atlantic salmon myofibrils and myosin from white and red muscles. Journal of Agricultural and Food Chemistry, 55:4761-4770.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Park, J.D., Yongsawatdigul, J., Choi, Y.J. and Park, J.W. 2008. Biochemical and conformational changes of myosin purified from Pacific sardine at various pHs. Journal of Food Science, 73:C191-C197.

Park, J.W. and Lanier, T.C. 1989. Scanning calorimetric behavior of tilapia myosin and actin due to processing of muscle and protein-purification. Journal of Food Science 54:49-51.

Poulter, R.G., Ledward, D.A., Godber, S., Hall, G. and Rowlands, B. 1985. Heat-stability of fish muscle proteins. Journal of Food Technology. 20:203-217.

Privalov, P.L. 1982. Stability of proteins - proteins which do not present a single cooperative system. Advances in Protein Chemistry, 35:1-104.

Riemann, A.E., Lanier, T.C. and Swartzel, K.R. 2004.

Rapid heating effects on gelation of muscle proteins. Journal of Food Science, 69: 308-314.

Saeed, S. and Howell, N.K. 2004. Rheological and differential scanning calorimetry studies on structural and textural changes in frozen Atlantic mackerel (*Scomber scombrus*). Journal of the Science of Food and Agriculture, 84:1216-1222.

Sano, T., Noguchi, S.F., Matsumoto, J.J. and Tsuchiya, T. 1990. Effect of ionic-strength on dynamic viscoelastic behavior of myosin during thermal gelation. Journal of Food Science, 55:51-54.

Sano, T., Noguchi, S.F., Tsuchiya, T. and Matsumoto, J.J. 1988. Dynamic viscoelastic behavior of natural actomyosin and myosin during thermal gelation. Journal of Food Science, 53:924-928.

Sano, T., Noguchi, S.F., Tsuchiya, T. and Matsumoto, J.J. 1989. Contribution of tropomyosin to fish muscle gel characteristics. Journal of Food Science 54:258-264.

Stone, A.P. and Stanley, D.W. 1992. Mechanisms of fish muscle gelation. Food Research International, 25:381-388.

Sun, X.D. and Arntfield, S.D. 2011. Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. Food Chemistry, 124:1011-1016.

Togashi, M., Kakinuma, M., Nakaya, M., Ooi, T. and Watabe, S. 2002. Differential scanning calorimetry and circular dichroism spectrometry of walleye pollack myosin and light meromyosin. Journal of Agricultural and Food Chemistry, 50:4803-4811.

Toyohara, H. and Shimizu, Y. 1988. Relation between the modori phenomenon and myosin heavy-chain breakdown in threadfin-bream gel. Agricultural and Biological Chemistry, 52:255-257.

Xiong, Y.L. and Blanchard, S.P. 1994. Myofibrillar protein gelation - viscoelastic changes related to heating procedures. Journal of Food Science, 59:734-738.

Yongsawatdigul, J. and Park, J.W. 1996. Linear heating rate affects gelation of Alaska pollock and Pacific whiting surimi. Journal of Food Science, 61:149-153.

Yongsawatdigul, J. and Park, J.W. 1999. Thermal aggregation and dynamic rheological properties of Pacific whiting and cod myosins as affected by heating rate. Journal of Food Science, 64:679-683.

Yongsawatdigul, J. and Park, J.W. 2003. Thermal denaturation and aggregation of threadfin bream actomyosin. Food Chemistry, 83:409-416.

Yongsawatdigul, J. and Piyadhammaviboon, P. 2004. Inhibition of autolytic activity of lizardfish surimi by proteinase inhibitors. Food Chemistry, 87:447-455.